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Quantifying Glomerular Filtration Rates in Acute Kidney Injury: A Requirement for Translational Success

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Abstract

Acute Kidney Injury (AKI) remains a vexing clinical problem resulting in unacceptably high patient mortality, development of chronic kidney disease and accelerated progression to end stage kidney disease. Although clinical risks factors for developing AKI have been identified, there is no reasonable surveillance technique to definitively and rapidly diagnose and determine the extent of severity of AKI in any patient. Since patient outcomes correlate with the extent of injury, and effective therapy likely requires early intervention, the ability to rapidly diagnose and stratify patients by their level of kidney injury is paramount for translational progress. Many groups are developing and characterizing optical measurement techniques utilizing novel minimally invasive or non-invasive techniques that can quantify kidney function, independent of serum or urinary measurements. Utilization of both one and two compartmental models, as well as continuous monitoring, are being developed. The purpose of this review is to document the need for GFR measurement in AKI patients and discuss the approaches being taken to deliver this overdue technique that is necessary to help propel nephrology to individualization of care and therapeutic success.

INTRODUCTION: The Clinical Problem

AKI remains an untreatable and deadly disease process affecting 5–10% of all hospitalized and 30–50%of ICU patients with a mortality rate often exceeding 50% in the ICU setting.[1–3] The rate of AKI in hospitalized patients has been increasing for over 30 years and yet the clinical approach has remained the same. Over this period there has been a marked increase in AKI, with the age adjusted rate increasing from 18 to 365 per 100,000 population.[1,2] AKI is associated with markedly increased mortality rates in numerous clinical situations including sepsis, following administration of radiocontrast dye and cardiovascular

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surgery.[4–8] Hsu et. al. quantified the increased incidence of non-dialysis and dialysis requiring AKI among members of an integrated health care delivery system (ref 8). In-hospital death rates were much higher in AKI discharges than in non AKI discharges. Using the data collected, it was also calculated that at least 22.4% of the end stage kidney disease (ESKD) cases in the United States come from Medicare beneficiaries with hospital acquired AKI. Numerous publications utilizing clinical scoring criteria to stratify patients into stages based on the maximum serum creatinine levels and urinary output showed outcomes including mortality, length of hospital stay (LOS), ICU length of stay, hospital costs, and the need for renal replacement therapy related to the highest stage reached in this stratification system.[9,10] These data indicate the severity of kidney injury is an outcomes prognostic indicator for both near and long terms. Data also implicated AKI as an initiator of chronic kidney disease (CKD) and the progression to end stage kidney disease (ESKD).[11] Therefore, AKI prevalence in hospitalized patients is increasing at an alarming rate, injury severity determines hospital outcomes, and AKI accelerates both the development of CKD and its progression to ESKD.

Given the lack of progress in AKI what will it take to turn the tide?

Of major importance is the advancement of therapeutic options to prevent or minimize the near term and/or long term consequences of AKI. Agents effective against many different plausible preclinical therapeutic targets have been developed and advanced into clinical trials.[12] Unfortunately, clinical trials in AKI have been unsuccessful and what was once seen as an attractive and large unmet need by pharmaceutical and biotech companies is now being viewed with skepticism, and some companies have even abandoned the area. Why the failure to translate highly effective preclinical therapies into effective clinical trials? This has often been attributed to inappropriate preclinical models and in response new models are being developed to more closely mimic the population being studied. The multifocal nature of AKI and the lack of human tissue to interrogate and compare to animal models at structural and molecular levels, as is being done in glomerular diseases [13], are also barriers to success. In this line of reasoning, individualization of therapy is not presently possible and likely won't be in the near term. However, we now have the ability to quantify GFR and I will argue this should serve as the first step in individualizing therapy and quantifying the response to therapy, as the most appropriate measure of therapeutic success.

QUANTIFYING GFR AS A MEASURE OF KIDNEY FUNCTION

Serum Creatinine and eGFR

GFR, which measures the amount of plasma filtered through glomeruli within a given period of time, is a physiologic process and as such a direct indicator of kidney function. It became the cornerstone of Nephrology beginning with Homer Smith and was actively measured in clinical studies in the past.[14] Unfortunately, the technique used then, inulin clearance, and the adaptations that followed remain cumbersome, time consuming and are not practical for studies in AKI for many reasons. Therefore, physicians base diagnostic and therapeutic decisions in millions of patients daily using a surrogate marker of GFR, that being the patient's serum creatinine concentration.[15,16]

While it is well known that reductions in the GFR, secondary to either acute or chronic kidney injury, are accompanied by increases in blood serum creatinine, the insensitivity of this surrogate marker as a measure of GFR is not uniformly appreciated. Neither serum creatinine nor one of several derived equations to estimate GFR (eGFR), based primarily on the serum creatinine, can be used in AKI, nor can they be used reliably over the range of GFRs.[17–19] One of the many reasons for this is illustrated in figure 1 where measured GFR vs serum creatinine (SCr) is shown. The graph is divided into insensitive, transition and sensitive regions based on the slope of the curve. In higher GFR regions of this curve it takes a very large change in GFR to result in even a small measureable change in SCr. To illustrate this point, consider what a change of 0.3 mg/dl of SCr means, calculated using standard eGFR formulas as is shown in figure 1b. In the insensitive region of the GFR-SCr curve, (SCr 1.0) a change of 0.3 represents a loss of over 30 ml/min of baseline GFR. In the sensitive portion of the curve (SCr 2.5 mg/dl) an increase of 0.3 SCr represents a loss of 5 ml/min. If one adds in the ability of the kidney to further increase GFR with stimuli such as a protein load or stress, the so called “renal reserve” or “augmented renal clearance”, then one can see why SCr or eGFR cannot provide an adequate measure of GFR change in this portion of the curve.

Renal reserve (RR) is that portion of GFR above baseline that is activated by stress, a protein load, amino acid infusion or glucagon infusion. It is felt to be proportionally lost as baseline GFR decreases, but very little is known about RR in CKD and aging patients. The most recent study showed no predictable effect on RR in patients with variable levels of GFR.[20] Chronic activation of RR occurs in diabetic patients and may portend a higher risk for progression to CKD and ESRD. It has also been show by many investigators to be activated in critical care situations where GFRs have been documented with daily 24 hr creatinine clearances for many days in patients on the ventilator and those receiving vasopressors for hemodynamic support.[21] There are no data regarding what happens in AKI, but it may well be that this portion of kidney function is activated and lost first prior to showing a loss in baseline GFR or a rise in SCr. In a patient with normal baseline GFR this means a change in SCr indicative of AKI would not occur until up to two thirds of the patient’s total GFR (baseline plus RR) was lost.

For example, take the transplant kidney donor studied prior to and post donation.[22] In this study baseline and stimulated GFRs were measured pre-donation and compared to post donation baseline values. Following donation serum creatinine increased from 0.96 +/− 0.15 to 1.29+/− 0.24 while baseline GFR decreased form 113 ml/min to 72 ml/min. The pre-donation stimulated GFR was 143ml/min. Therefore, loss of one kidney, or 50% of total GFR, resulted in only an increase of 0.33 mg/dl in SCr, a loss of 41 ml/min of baseline GFR, but a loss of 71ml/min total GFR. This was exactly half of the total pre-donation stimulated or total GFR implying total engagement of the renal reserve post donation. At one year of follow up the SCr was unchanged. This also brings home the point that a 0.3mg/dl change in SCr in a patient with normal kidney function represents a major insult in overall kidney function. This may explain why AKI in patients with near normal GFRs have an increased hospital mortality compared to patients with CKD where a less severe injury results in an adequate SCr readout to document AKI.[8] It may also explain why some patients with AKI according to urinary biomarkers, but without an elevation in SCr, have higher morbidity and

mortality than those with negative biomarkers and SCr.[23] This so called "subclinical AKI" because of the insensitivity of SCr, is now well documented and represents a clear advantage of new urinary biomarkers over SCr. Clearly, an injury resulting in the loss of 25ml/min GFR in a patient with normal kidney function could have less than a 0.3 mg/dl change in SCr and yet have positive urinary biomarkers. This would be unlikely in a patient with CKD where the loss of a few ml/min can be detected by a rise in SCr (Figure 1b).

The discrepancy between what an increase of 0.3mg/dl SCr means at different starting SCr brings up another important point, especially when one considers that all therapeutic trials in AKI are done on patients with CKD in order to increase the likelihood of "AKI events" in the study population. What this means is that clinical trialists have shifted the study population to those giving a large change in SCr for a small change in GFR to enhance their ability to detect the signal. Thus, patients with a 5–7 ml/min or less loss of GFR are studied rather than patients starting with a more normal GFR that would have to have a far larger loss of GFR due to the insensitivity of SCr. This is compounded when one considers renal reserve activation during AKI. Given that one of the therapeutic end points for registration of the drug is a permanent loss of GFR at 90 days is this a wise approach? Can we trust SCr to deliver on this endpoint? The standard techniques presently used to quantify GFR have coefficients of variability greater than the 5–7 ml/min and with a change of SCr of only 0.3 in patients with CKD one is looking to register a drug on saving 1–2 ml/min (25% of 5–7ml/min). Thus, one needs to consider this factor when designing studies for drug registration. Also, one has to ask if the drug will work in CKD patients for two reasons. First, likely it was only profiled for effectiveness in normal animals in preclinical studies. Second, the underlying process may already be in a stage where no therapy will work given the endothelial and tubular epithelial changes that occur in the later stages of CKD.

Other factors limit the ability of SCr to be used in AKI. Since both muscle mass (creatinine comes from creatinine released from muscle) and GFR determine a patient's serum creatinine level, using serum creatinine as an indicator of GFR is highly patient-specific. For instance, a serum creatinine of 1.0 mg/dl is indicative of normal GFR (100ml/min/1.73 m^2) in a 70 Kg male with normal muscle mass. However, in a 50 Kg male with muscle wasting a serum creatinine of 1.0 mg/dl is seen often with a GFR of only 50 ml/min. Therefore, formulas derived from large population studies have been developed to factor in patient weight, age, sex and race. However, these formulas are often inaccurate and misleading[24–29] This inaccuracy was also documented for creatinine clearances.[30,31] For AKI in particular, this may relate to the known reduced production of creatinine in sepsis, at least in preclinical models.[32]

Finally, even in situations where SCr is stable the estimating equations have major limitations. In a recent review it was stated that "In usual practice, an eGFR equation is defined as having sufficient accuracy when at least 75% of the estimates fall within +/− 30% of the measured GFR.[33] This means an eGFR of 50 indicates the patient's measured GFR would fall between 65 and 35ml/min 75% of the time, and one in four patients would fall outside of this range. The clinical importance of this was recently reviewed.[33] eGFR formulas are also difficult to use in population groups outside of the study population used for their derivation.[27,28]

Renal reserve and muscle mass are also important factors in following a patient's recovery with regard to permanent loss of kidney function. In a recent article Prowle and co-authors followed 700 patients with a 66% incidence of AKI following an ICU admission of five or more days.[34] In these patients SCr led to an inaccurate and over estimate of kidney function in all patients, those with and without AKI. Even in patients with KDIGO stage III AKI no permanent loss of kidney function could be demonstrated at follow up. This was likely the result of muscle wasting, decompensation and likely the engagement of renal reserve. However, this was not determined. Very concerning is the "apparent" return to baseline kidney function, based on SCr or eGFR, following various levels of AKI including stage three in patients starting with normal or near normal function prior to the AKI event. How can a therapeutic be successful in this population given these results?

In addition, neither SCr nor eGFRs can be used in the setting of evolving or recovering AKI because of the time it takes to reach equilibrium creatinine values which are required for an accurate conversion. For example, although patients with acute kidney injury may develop an abrupt GFR decline the magnitude of this decline is only apparent after several days of equilibration if determined using a rising serum creatinine. If a patient was to lose 95% of their GFR secondary to AKI, the GFR would decrease from 100 to 5 ml/min rapidly but the serum creatinine would only rise by 1–2 mg/dl/day. This slow rise in serum creatinine eliminates the ability to diagnose the injury rapidly and it is also not possible to determine the total extent of injury for days. To find a way around this two studies have used modeling to both redefine AKI (Waikar and Bonventre JASN 20: 672, 2009) and estimate a "kinetic GFR" when plasma creatinine is changing (Chen, S. JASN 24:877, 2013) However, the kinetic estimation of GFR required determination of the volume of distribution, creatinine production rate and quantitative difference between consecutive plasma creatinines over a given time when the plasma creatinine was changing. This does not seem practical in the clinical setting.

Since the extent of decline in GFR, or eventual plateau in serum creatinine, correlates with morbidity, mortality and the potential for recovery [9,10,35,36] accurately determining GFR in patients with AKI is of great clinical importance including rapid diagnosis, stratification, and timely treatment. The inability to do this has markedly limited the ability to conduct a successful therapeutic trial in AKI. It is widely held that beginning therapy after 12 – 24 hours of AKI may limit the success rate of any potential therapeutic agent. Therefore, a search for a diagnostic biomarker of kidney injury is now considered by many experts to be the highest priority in the AKI field.[16,37,38] However, the extent of injury, rate of recovery, response to therapy and the magnitude of recovery are not approachable utilizing presently available structural biomarkers and a combination of both structural and functional determinants of AKI will be synergistic.

Determining Glomerular Filtration Rate

The rapid and accurate clinical determination of GFR has been a clinical goal for over fifty years. The clinical utility for a measured GFR (mGFR) is listed in Table 1. Collection of a 24 hour urine and invasive techniques exist to approximate a patient's GFR, but these approaches are cumbersome, error prone, expensive, time consuming, and can expose the

patient to potential harm including radiation or radiocontrast media.[15] There is also not a rapid and accurate technique to determine GFR reliably in patients with AKI when the serum creatinine is rising or falling. Since release of creatinine by muscles is not consistent throughout the day, and is affected by acute diseases, short term urine collections can be misleading.[31] To measure GFR accurately the ideal GFR marker should be small, retained within the vasculature, not protein bound, and freely filtered across the glomerulus. It should not be secreted into the urine, reabsorbed from the urine, or metabolized within the kidney so that the measured GFR would be equal to the urinary clearance of the marker after its intravenous infusion. Inulin, a small fructose polymer that is neither secreted, reabsorbed, nor metabolized, and cleared only by glomerular filtration, is the reference standard GFR marker. However, it is not retained exclusively within the intravascular volume as it also distributes into the interstitial volume to occupy the total extracellular fluid volume (ECF). Therefore, the disappearance of inulin, and other small molecular weight compounds used to quantify GFR from the intravascular volume, is governed by both interstitial space distribution and kidney clearance. This results in early rapid vascular clearance, due to interstitial redistribution, and kidney removal of the compound. To remove the interstitial distribution component from the equation the terminal elimination phase k constant is used as it relates directly to kidney removal of the compound. At this time the vascular and interstitial concentration of the GFR reporter are identical. This is why the one compartment model requires several hours of recurrent blood draws for an accurate determination. Other non-radioactive markers (such as iothalamate, iohexol, and polyfructosan) and radioactive ones (such as ^{125}I-iothalamate and 51Cr-EDTA) are used for such studies. Radioactive GFR markers, such as ^{39} Cr-EDTA and 99mTc-DTPA (99m-Technetium diethylene triamine pentaacetate) have been used in conjunction with a radiation detector to monitor GFR in patients with AKI at rates close to real-time.[40,41] The measured plasma clearance showed excellent correlations with GFRs simultaneously measured using the standard method with urine collection.[40,41] However, the use of radioactive GFR markers makes this method not attractive for obvious reasons, and Cr-EDTA cannot be used in the United States. By using a fluorescent GFR marker, such as FITC-inulin, with a bolus intravenous infusion followed with drawing blood samples at multiple time points, one can accurately determine a clearance rate constant and, if plasma volume is known, a measured GFR.[42–45] Medical imaging with the development of a suitable contrast agent, for instance magnetic resonance imaging (MRI) techniques, will be useful for providing kidney functional diagnostics.[46–49] The downside of using such technologies is the low accessibility, associated high cost, difficulty repeating the study, and the need to transport the patient for the study.

In summary, the rapid and accurate measurement of GFR in AKI is important for diagnosis, severity of injury stratification, and potential therapeutic purposes. Rapid identification and determination of the extent of AKI will allow for early treatment, including dialysis initiation, as well as enrollment and stratification in clinical studies. It could also be used to determine the effect of a clinical maneuver on GFR such as volume resuscitation, and the loss of baseline and or total GFR subsequent to the injury event. Therefore, this would represent a technical advance of major clinical importance, especially in high risk patients.

Translating Fluorescence Measurements into Clinical Observations Progress in the Area of Fluorescence Tissue Imaging

Since the beginning of 1990, the idea of using visible or near infrared (IR) light to image live tissue has been gradually developed in both theory and in practice. According to photon diffusion theories (also known as diffusive photon or photon migration) which consider the non-coherent property or the wave nature of photons[50–53], one can detect photons injected into the tissue at a relatively large distance (up to 10 cm and beyond) away from the light source.[52,54,55] Similarly, one can also detect fluorescence photons emitted from the tissue by delivering excitation light into the tissue at a distance away from the detectors.[51,56–61] Tissue properties can be studied and measured using photon diffusion technologies.[39,62,63] Intrinsically, optical techniques that use non-ionizing radiation are either minimally- or non-invasive. They can empower the diagnosis of diseases with high sensitivity, speed and accuracy.

Two-photon fluorescence microscopy is a promising technique especially for imaging of highly scattering biological specimens.[64–69] With this technique ultrafast near-infrared pulse lasers provide long wavelength low energy photons used as the excitation light source allowing for deeper tissue penetration than visible and ultraviolet light. The benefits of using two-photon excitation for viable tissue imaging have been studied and reviewed.[70–72] This imaging technique holds particular promise for studying kidney functions as it allows direct observations of local regions of the kidney, such as the glomerulus, proximal tubule cells, and distal tubule cells, with subcellular and sub-micron resolution and at the same time with nanomolar to micromolar concentration sensitivity.[67,73–77] In the past few years significant progress has been made in using two-photon excitation fluorescence microscopy to study kidney function and dysfunction by imaging live animals.[65,67–69,75–82] In particular, quantitative fluorescent approaches have been developed for determining numerous kidney functions including GFR.[65,67–69,75–85] Several groups of investigators are working on this same idea as is shown by recent publications (table 2).[83,86–93]

Initial studies in mice showed a single injection of FITC-inulin could be used to quantify GFR with normal, reduced and increased GFR.[43] Continuous infusions of FITC-inulin have also been used with success in mice [94] to quantify hyperfiltration in diabetic mice. Sinistrin, a fluorescent derivative of inulin that has increased solubility, has also been used in bolus and continuous infusion studies in rats.[90,91,95] These later studies were accomplished using a transcutaneous monitor of fluorescence reading the ECF fluorescence. Sinistrin has also been used in humans with variable levels of kidney function including patients with augmented renal clearance.[96,97] Other dyes have also been studied in rodent models and lifetime-decomposition measurements of FITC-sinistrin have been used to quantify GFR.[89,98] The use of lifetime analysis allowed a reduction in sinistrin dose by factor of 200. However, all of these studies had to use a single compartment model as the fluorescent signal comes for the ECF. This approach requires equilibration of the injected fluorescent marker with the ECF and increases the amount of marker needed for adequate signals. It also requires an estimate of the plasma volume (PV) as actual concentrations of the markers are not determined. Thus, a rate constant is determined for removal of the substance from the

plasma and the rate constant must be multiplied by an estimated PV. As multiple factors affect PV an estimate based on normal physiology may give misleading results.

Developing a Bedside Measured Two-Compartment mGFR Technique

Translation of fluorescent technology into quantitative in vivo clinical applications has been rare due to its complex and platform-dependent nature.[99] As a result accurate determination of the concentration of fluorescent molecules under in vivo conditions is usually very difficult. Translation requires refining an approach initially developed in rats that would allow rapid direct quantification of vascular fluorescence for quantitative analysis of GFR and plasma volume.[83] Three components are necessary to translate from the microscope to the bedside. These include a two component fluorescent injectate consisting of a high molecular weight PV indicator and a small molecular weight glomerular filtration indicator. To measure GFR accurately the ideal GFR plasma volume marker molecule should be retained within the vasculature, not be metabolized within the vascular compartment during the study, have a glomerular sieving coefficient (GSC) of 0.0, and it should not be secreted by the kidney. The ideal GFR filtration reporter molecule is freely filterable by the kidney (GSC of 1.0), does not bind to other plasma constituents, is not secreted by the kidney, and does not undergo metabolism during the procedure. Following a bolus infusion of GFR reporter molecules the plasma concentration of these molecules decreases as a function of time and is dependent upon both kidney clearance and redistribution within the vasculature and into the extra cellular fluid (ECF). The large fluorescent PV marker molecule serves to quantify the plasma volume of distribution based on principles of dilution. It should be large enough not to “leak” into the ECF, even in disease states, to give reliable readouts during the timeframe of the study.

Dextrans are inert and highly water soluble molecules that can be correctly sized and have been used clinically for many decades. They are also easy to label with fluorescent molecules with a high conjugation ratio. These characteristics allow for very low dose volumes and mass to be given. This allows for very small amounts of fluorescent dextrans to be used in studies and has important clinical safety and financial implications. In addition, the use of one fluorescent dextran as a PV marker, and a second freely filterable small highly fluorescent reporter molecule to quantify the rate of plasma clearance as an assay for GFR, can replace the necessity of concentration measurements of the filtration reporter with relative fluorescence intensity determinations. The relatively stable and impermeable nature of the large marker molecule allows for accessing the plasma volume directly thus minimizing potential errors due to variations in PV with disease states and inaccurate estimates of time zero concentrations. Use of a large PV indicator such as 150kDa dextran molecule avoids movement of the molecule into the extracellular space especially in disease states like sepsis thus allowing for a stable measurement over time. Purity, in terms of the size distribution or molecular weight of the dextran molecules, is critical. The distribution of molecular weight about the mean weight plays an important role in how accurately GFR can be determined. Even though dextrans are widely used in medical applications, these previous applications did not require the stringent size control needed for use with the present technique.

The second component necessary for a rapid bedside determination is a two compartment model. Using a two compartment mathematical model one can reduce the time it takes to determine a GFR compared to a one compartment model. The GFR rate constant, and apparent volume of distribution (V_d) of the PV indicator molecule, can be measured by monitoring the plasma concentration of the fluorescently labeled GFR filtration molecule over time and dilution equilibrium value of the PV indicator molecule. The two compartments in question are the vascular and interstitial spaces. The basic assumption for this model is that after the bolus injection the infused filtration molecule, but not the larger PV indicator molecule, will distribute from the vascular space to interstitial space, but true plasma clearance by the kidney only occurs from the vascular space.

The plasma clearance rate and the inter-compartment clearance rate are denoted as G and α, respectively. The virtual volume for the vascular and interstitial spaces are V_1 and V_2, respectively. The two-compartment pharmacokinetics model, as described previously,[44,83,100–102] has been extensively used for determination of GFR. The conventional single compartment approach to determine GFR requires determinations of the plasma concentrations of the filtration reporter from multiple blood samples drawn over 5–6 hours as the early phase rapid decay makes it impossible to properly determine the T0 point using only the curve fit of later sample times. Thus, single compartment models rely on much longer test times to compensate for missing this early data. As was demonstrated recently,[83,92] both requirements can be eliminated by using the two-compartment model. We applied this approach to studies in large animals using the optical fiber device.[92] Following bolus injection the infused smaller dextran molecules were cleared by the kidneys and distributed from the vascular into the interstitial space, but did not move intracellularly; the large dextran molecules were retained in the vascular space during the course of the study. Based on the principle of dilution, a true vascular volume can be calculated from a 10 minute blood draw. Using the measured vascular volume and known dose, a virtual T0 point can be accurately calculated. The concentration of the freely kidney filterable reporter (5 kDa fluorescein-dextran) over time determined by the background-corrected fluorescence, including the derived T0 point, was fit to a bi-exponential equation (Eq.1) using the Sigma plot program to obtain the amplitudes and decay constants:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C_t was the concentration in the plasma at time, t, A and B are the initial amplitudes, and α and β are the decay rates. GFR, G, was then calculated using the equation:

$$G = \frac{D\alpha\beta}{A\beta + B\alpha} = \frac{D}{\frac{A}{\alpha} + \frac{B}{\beta}} \quad (2)$$

Since its introduction,[102] the two-compartment model has been applied in a number of renal studies on animal subjects [43,45,101] as well as on human subjects.[100,103,104] It has been shown in these studies to be an effective approach in plasma clearance analysis and GFR determination.

When the inter-compartment volume exchange rate approaches 0, this model collapses to a single compartment model. Thomaseth and Amici [105] used this simplified model to measure plasma clearance of ^{125} I iothalamate for GFR and V_d assessment on patients under peritoneal dialysis (PD).

The third and final component is a fluorescent detector including software for reading, analyzing, and reporting data, a user interface to control the apparatus and view results, and the ability to send and receive fluorescent signals. Plasma samples, from timed blood draws, are placed into the instrument and analyzed for the fluorescence intensity. The fluorescence characteristics of the molecules make them easy to read compared to other types of GFR markers. The main difference between single channel designs and the two channel design is an additional dichroic filter within holders, separated from the main block by spacers used to combine light from the 490 nm LED and 595 nm LED together. This set-up allows for both molecules to be read simultaneously on each sample, decreasing overall sample processing time and instrument size.

A rapid determination of GFR has multiple advantages. First, it can be used for bedside determinations in the hospital where rapid, 10 minutes for PV and less than 120 minutes for GFR, are necessary for clinical decisions. This approach also allows for a shortened time for outpatient determinations. Finally, it allows for measurement of renal reserve and therefore total GFR. To accomplish this either an IV infusion of an amino acid mixture or a protein meal can be used.[106,107] Using a two compartment model this can be done either following a baseline measurement of GFR by repeating the study post stimulation or without a baseline measurement by a GFR study one hour post stimulation. The former allows for determination of both baseline GFR, and total GFR with the difference being renal reserve. Not known is whether measuring the total GFR will allow for more sensitive and accurate measurements of kidney function and its loss during disease. Measuring renal reserve would also likely give early insight into hyperfiltration in diabetics allowing for earlier initiation of therapies to reduce intraglomerular pressure.

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References

1. Hospitalization discharge diagnosis for kidney disease - United States, 1980–2005. MMWR Morb Mortal Wkly Rep. 2008; 57:309–12. [PubMed: 18368005]
2. Palevsky PM. Setting the agenda. Acute kidney injury Clinical journal of the American Society of Nephrology : CJASN. 2008; 3:933–4. [PubMed: 18579670]
3. Waikar SS, Liu KD, Chertow GM. Diagnosis, epidemiology and outcomes of acute kidney injury. Clinical journal of the American Society of Nephrology : CJASN. 2008; 3:844–61. [PubMed: 18337550]

4. Chertow GM, Levy EM, Hammermeister KE, Grover F, Daley J. Independent association between acute renal failure and mortality following cardiac surgery. The American journal of medicine. 1998; 104:343–8. [PubMed: 9576407]

5. Levy EM, Viscoli CM, Horwitz RI. The effect of acute renal failure on mortality. A cohort analysis. JAMA : the journal of the American Medical Association. 1996; 275:1489–94. [PubMed: 8622223]

6. Newsome BB, Warnock DG, McClellan WM, et al. Long-term risk of mortality and end-stage renal disease among the elderly after small increases in serum creatinine level during hospitalization for acute myocardial infarction. Archives of internal medicine. 2008; 168:609–16. [PubMed: 18362253]

7. Bagshaw SM, George C, Bellomo R, Committee ADM. Early acute kidney injury and sepsis: a multicentre evaluation. Critical care. 2008; 12:R47. [PubMed: 18402655]

8. Hsu CY, McCulloch CE, Fan D, Ordonez JD, Chertow GM, Go AS. Community-based incidence of acute renal failure. Kidney international. 2007; 72:208–12. [PubMed: 17507907]

9. Hoste EA, Clermont G, Kersten A, et al. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. Critical care. 2006; 10:R73. [PubMed: 16696865]

10. Uchino S, Bellomo R, Goldsmith D, Bates S, Ronco C. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. Critical care medicine. 2006; 34:1913–7. [PubMed: 16715038]

11. Chawla LS, Amdur RL, Amodeo S, Kimmel PL, Palant CE. The severity of acute kidney injury predicts progression to chronic kidney disease. Kidney international. 2011; 79:1361–9. [PubMed: 21430640]

12. Faubel S, Chawla LS, Chertow GM, et al. Ongoing clinical trials in AKI. Clinical journal of the American Society of Nephrology : CJASN. 2012; 7:861–73. [PubMed: 22442183]

13. Kretzler M, Sedor JR. Introduction: Precision Medicine for Glomerular Disease: The Road Forward. Semin Nephrol. 2015; 35:209–11. [PubMed: 26215858]

14. Finkenstaedt JT, Merrill JP. Renal function after recovery from acute renal failure. The New England journal of medicine. 1956; 254:1023–6. [PubMed: 13322205]

15. Dagher PC, Herget-Rosenthal S, Ruehm SG, et al. Newly developed techniques to study and diagnose acute renal failure. Journal of the American Society of Nephrology : JASN. 2003; 14:2188–98. [PubMed: 12874475]

16. Zhou H, Hewitt SM, Yuen PS, Star RA. Acute Kidney Injury Biomarkers - Needs, Present Status, and Future Promise. Nephrology self-assessment program : NephSAP. 2006; 5:63–71. [PubMed: 19096722]

17. Froissart M, Rossert J, Jacquot C, Paillard M, Houillier P. Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function. Journal of the American Society of Nephrology : JASN. 2005; 16:763–73. [PubMed: 15659562]

18. Poggio ED, Wang X, Greene T, Van Lente F, Hall PM. Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. Journal of the American Society of Nephrology : JASN. 2005; 16:459–66. [PubMed: 15615823]

19. Stevens LA, Levey AS. Clinical implications of estimating equations for glomerular filtration rate. Annals of internal medicine. 2004; 141:959–61. [PubMed: 15611494]

20. Thomas DM, Coles GA, Williams JD. What does the renal reserve mean? Kidney international. 1994; 45:411–6. [PubMed: 8164427]

21. Baptista JP, Udy AA, Sousa E, et al. A comparison of estimates of glomerular filtration in critically ill patients with augmented renal clearance. Critical care. 2011; 15:R139. [PubMed: 21651804]

22. Rook M, Hofker HS, van Son WJ, Homan van der Heide JJ, Ploeg RJ, Navis GJ. Predictive capacity of pre-donation GFR and renal reserve capacity for donor renal function after living kidney donation. Am J Transplant. 2006; 6:1653–9. [PubMed: 16827867]

23. Haase M, Kellum JA, Ronco C. Subclinical AKI--an emerging syndrome with important consequences. Nat Rev Nephrol. 2012; 8:735–9. [PubMed: 23007617]

24. Botev R, Mallie JP, Couchoud C, et al. Estimating glomerular filtration rate: Cockcroft-Gault and Modification of Diet in Renal Disease formulas compared to renal inulin clearance. Clinical journal of the American Society of Nephrology : CJASN. 2009; 4:899–906. [PubMed: 19406960]
25. Botev R, Mallie JP, Wetzels JF, Couchoud C, Schuck O. The clinician and estimation of glomerular filtration rate by creatinine-based formulas: current limitations and quo vadis. Clinical journal of the American Society of Nephrology : CJASN. 2011; 6:937–50. [PubMed: 21454722]
26. Gaspari F, Ruggenenti P, Porrini E, et al. The GFR and GFR decline cannot be accurately estimated in type 2 diabetics. Kidney international. 2013; 84:164–73. [PubMed: 23447062]
27. Hingorani S, Pao E, Schoch G, Gooley T, Schwartz GJ. Estimating GFR in adult patients with hematopoietic cell transplant: comparison of estimating equations with an iohexol reference standard. Clinical journal of the American Society of Nephrology : CJASN. 2015; 10:601–10. [PubMed: 25717073]
28. Murata K, Baumann NA, Saenger AK, Larson TS, Rule AD, Lieske JC. Relative performance of the MDRD and CKD-EPI equations for estimating glomerular filtration rate among patients with varied clinical presentations. Clinical journal of the American Society of Nephrology : CJASN. 2011; 6:1963–72. [PubMed: 21737852]
29. Rule AD, Glassock RJ. GFR estimating equations: getting closer to the truth? Clinical journal of the American Society of Nephrology : CJASN. 2013; 8:1414–20. [PubMed: 23704300]
30. Bragadottir G, Redfors B, Ricksten SE. Assessing glomerular filtration rate (GFR) in critically ill patients with acute kidney injury--true GFR versus urinary creatinine clearance and estimating equations. Critical care. 2013; 17:R108. [PubMed: 23767877]
31. Cherry RA, Eachempati SR, Hydo L, Barie PS. Accuracy of short-duration creatinine clearance determinations in predicting 24-hour creatinine clearance in critically ill and injured patients. The Journal of trauma. 2002; 53:267–71. [PubMed: 12169932]
32. Doi K, Yuen PS, Eisner C, et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. Journal of the American Society of Nephrology : JASN. 2009; 20:1217–21. [PubMed: 19389851]
33. Berns JS. Clinical Decision Making in a Patient with Stage 5 CKD-Is eGFR Good Enough? Clinical journal of the American Society of Nephrology : CJASN. 2015; 10:2065–72. [PubMed: 25883071]
34. Prowle JR, Kolic I, Purdell-Lewis J, Taylor R, Pearse RM, Kirwan CJ. Serum creatinine changes associated with critical illness and detection of persistent renal dysfunction after AKI. Clinical journal of the American Society of Nephrology : CJASN. 2014; 9:1015–23. [PubMed: 24742481]
35. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. Journal of the American Society of Nephrology : JASN. 2005; 16:3365–70. [PubMed: 16177006]
36. Lassnigg A, Schmidlin D, Mouhieddine M, et al. Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. Journal of the American Society of Nephrology : JASN. 2004; 15:1597–605. [PubMed: 15153571]
37. Hewitt SM, Dear J, Star RA. Discovery of protein biomarkers for renal diseases. Journal of the American Society of Nephrology : JASN. 2004; 15:1677–89. [PubMed: 15213255]
38. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:13368–73. [PubMed: 15326289]
39. Roy R, Sevick-Muraca E. Truncated Newton's optimization scheme for absorption and fluorescence optical tomography: Part I theory and formulation. Optics express. 1999; 4:353–71. [PubMed: 19396292]
40. Rabito CA, Moore RH, Bougas C, Dragotakes SC. Noninvasive, real-time monitoring of renal function: the ambulatory renal monitor. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 1993; 34:199–207.
41. Rabito CA, Panico F, Rubin R, Tolkoff-Rubin N, Teplick R. Noninvasive, real-time monitoring of renal function during critical care. Journal of the American Society of Nephrology : JASN. 1994; 4:1421–8. [PubMed: 8161724]

42. Greenblatt DJ, K-WJ. Clinical pharmacokinetics (first of two parts). The New England journal of medicine. 1973; 293(19):702–5. [PubMed: 1160938]
43. Qi Z, Whitt I, Mehta A, et al. Serial determination of glomerular filtration rate in conscious mice using FITC-inulin clearance. American journal of physiology Renal physiology. 2004; 286:F590–6. [PubMed: 14600035]
44. Sapirstein LA, Herrold MR, Janakis M, Ogden E. Validity of values for glomerular filtration rate and extracellular fluid obtained from plasma concentration-time decay curves after single injections of mannitol in the dog. The American journal of physiology. 1952; 171:487–91. [PubMed: 13007821]
45. Sturgeon C, Sam AD 2nd, Law WR. Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. Journal of applied physiology. 1998; 84:2154–62. [PubMed: 9609812]
46. Annet L, Hermoye L, Peeters F, Jamar F, Dehoux JP, Van Beers BE. Glomerular filtration rate: assessment with dynamic contrast-enhanced MRI and a cortical-compartment model in the rabbit kidney. Journal of magnetic resonance imaging : JMRI. 2004; 20:843–9. [PubMed: 15503326]
47. Buonocore MH, Katzberg RW. Estimation of extraction fraction (EF) and glomerular filtration rate (GFR) using MRI: considerations derived from a new Gd-chelate biodistribution model simulation. IEEE transactions on medical imaging. 2005; 24:651–66. [PubMed: 15889552]
48. Prasad PV. Functional MRI of the kidney: tools for translational studies of pathophysiology of renal disease. American journal of physiology Renal physiology. 2006; 290:F958–74. [PubMed: 16601297]
49. Rusinek H, Kaur M, Lee VS. Renal magnetic resonance imaging. Current opinion in nephrology and hypertension. 2004; 13:667–73. [PubMed: 15483459]
50. Chance B. Time resolved spectroscopic (TRS) and continuous wave spectroscopic (CWS) studies of photon migration in human arms and limbs. Advances in experimental medicine and biology. 1989; 248:21–31. [PubMed: 2782147]
51. Fantini SGE. Fluorescence photon-density waves in optically diffusive media. Optics Communications. 2000; 173(1–6):73–9.
52. Fishkin JBGE. Propagation of photon-density waves in strongly scattering media containing an absorbing semi-infinite plane bounded by a straight edge. Journal of the Optical Society of America A, Optics and image science. 1993; 10(1)
53. Schmitt JM, Knuttel A, Knutson JR. Interference of diffusive light waves. Journal of the Optical Society of America A, Optics and image science. 1992; 9:1832–43. [PubMed: 1403242]
54. Sevick EM, Burch CL, Chance B. Near-infrared optical imaging of tissue phantoms with measurement in the change of optical path lengths. Advances in experimental medicine and biology. 1994; 345:815–23. [PubMed: 8079791]
55. Venugopalan VYJ, Tomberg BJ. Radiative transport in the diffusion approximation: An extension for highly absorbing media and small source-detector seperations. Physical Review E. 1998; 58(2): 2395–407.
56. Cerussi AE, Maier JS, Fantini S, Franceschini MA, Mantulin WW, Gratton E. Experimental verification of a theory for the time-resolved fluorescence spectroscopy of thick tissues. Applied optics. 1997; 36:116–24. [PubMed: 18250652]
57. Godavarty A, Thompson AB, Roy R, et al. Diagnostic imaging of breast cancer using fluorescence-enhanced optical tomography: phantom studies. Journal of biomedical optics. 2004; 9:488–96. [PubMed: 15189086]
58. Lee J, Sevick-Muraca E. Fluorescence-enhanced absorption imaging using frequency-domain photon migration: tolerance to measurement error. Journal of biomedical optics. 2001; 6:58–67. [PubMed: 11178581]
59. Sevick-Muraca EM, Houston JP, Gurfinkel M. Fluorescence-enhanced, near infrared diagnostic imaging with contrast agents. Current opinion in chemical biology. 2002; 6:642–50. [PubMed: 12413549]
60. Thompson AB, Hawrysz DJ, Sevick-Muraca EM. Near-infrared fluorescence contrast-enhanced imaging with area illumination and area detection: the forward imaging problem. Applied optics. 2003; 42:4125–36. [PubMed: 12868856]

61. Wu J, Perelman L, Dasari RR, Feld MS. Fluorescence tomographic imaging in turbid media using early-arriving photons and Laplace transforms. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94:8783–8. [PubMed: 9238055]
62. Bevilacqua F, Piguet D, Marquet P, Gross JD, Tromberg BJ, Depeursinge C. In vivo local determination of tissue optical properties: applications to human brain. Applied optics. 1999; 38:4939–50. [PubMed: 18323984]
63. Fishkin JB, Coquoz O, Anderson ER, Brenner M, Tromberg BJ. Frequency-domain photon migration measurements of normal and malignant tissue optical properties in a human subject. Applied optics. 1997; 36:10–20. [PubMed: 18250643]
64. Denk W, Strickler JH, Webb WW. Two-photon laser scanning fluorescence microscopy. Science. 1990; 248:73–6. [PubMed: 2321027]
65. Dunn KW, Sandoval RM, Kelly KJ, et al. Functional studies of the kidney of living animals using multicolor two-photon microscopy. American journal of physiology Cell physiology. 2002; 283:C905–16. [PubMed: 12176747]
66. Larson DR, Zipfel WR, Williams RM, et al. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science. 2003; 300:1434–6. [PubMed: 12775841]
67. Molitoris BA, Sandoval RM. Intravital multiphoton microscopy of dynamic renal processes. American journal of physiology Renal physiology. 2005; 288:F1084–9. [PubMed: 15883167]
68. Molitoris BA, Sandoval RM. Pharmacophotonics: utilizing multi-photon microscopy to quantify drug delivery and intracellular trafficking in the kidney. Advanced drug delivery reviews. 2006; 58:809–23. [PubMed: 17064810]
69. Peti-Peterdi J, Fintha A, Fuson AL, Tousson A, Chow RH. Real-time imaging of renin release in vitro. American journal of physiology Renal physiology. 2004; 287:F329–35. [PubMed: 15082450]
70. Gratton E, Barry NP, Beretta S, Celli A. Multiphoton fluorescence microscopy. Methods. 2001; 25:103–10. [PubMed: 11559001]
71. Squier JMM. High resolution nonlinear microscopy: A review of sources and methods for achieving optimal imaging. Review of Scientific Instruments. 2001; 72(7):2855–67.
72. Williams RM, Zipfel WR, Webb WW. Multiphoton microscopy in biological research. Current opinion in chemical biology. 2001; 5:603–8. [PubMed: 11578936]
73. Centonze VE, White JG. Multiphoton excitation provides optical sections from deeper within scattering specimens than confocal imaging. Biophysical journal. 1998; 75:2015–24. [PubMed: 9746543]
74. Peti-Peterdi J. Multiphoton imaging of renal tissues in vitro. American journal of physiology Renal physiology. 2005; 288:F1079–83. [PubMed: 15883166]
75. Peti-Peterdi J, Morishima S, Bell PD, Okada Y. Two-photon excitation fluorescence imaging of the living juxtaglomerular apparatus. American journal of physiology Renal physiology. 2002; 283:F197–201. [PubMed: 12060602]
76. Phillips C. Cell of the month: waves of glomerular podocytes. Nature cell biology. 2004; 6:818. [PubMed: 15340449]
77. Tanner GA, Sandoval RM, Dunn KW. Two-photon in vivo microscopy of sulfonefluorescein secretion in normal and cystic rat kidneys. American journal of physiology Renal physiology. 2004; 286:F152–60. [PubMed: 12965895]
78. Dunn KW, Sandoval RM, Molitoris BA. Intravital imaging of the kidney using multiparameter multiphoton microscopy. Nephron Experimental nephrology. 2003; 94:e7–11. [PubMed: 12806182]
79. Lin SX, Maxfield FR. Fluorescence imaging in living animals. Focus on “Uptake and trafficking of fluorescent conjugates of folic acid in intact kidney determined using intravital two-photon microscopy” American journal of physiology Cell physiology. 2004; 287:C257–9. [PubMed: 15238357]
80. Sandoval RM, Kennedy MD, Low PS, Molitoris BA. Uptake and trafficking of fluorescent conjugates of folic acid in intact kidney determined using intravital two-photon microscopy. American journal of physiology Cell physiology. 2004; 287:C517–26. [PubMed: 15102609]

81. Sutton TA, Fisher CJ, Molitoris BA. Microvascular endothelial injury and dysfunction during ischemic acute renal failure. Kidney international. 2002; 62:1539–49. [PubMed: 12371954]
82. Tanner GA, Sandoval RM, Molitoris BA, Bamburg JR, Ashworth SL. Micropuncture gene delivery and intravital two-photon visualization of protein expression in rat kidney. American journal of physiology Renal physiology. 2005; 289:F638–43. [PubMed: 15886277]
83. Wang E, Sandoval RM, Campos SB, Molitoris BA. Rapid diagnosis and quantification of acute kidney injury using fluorescent ratio-metric determination of glomerular filtration rate in the rat. American journal of physiology Renal physiology. 2010; 299:F1048–55. [PubMed: 20685826]
84. Yu W. Quantitative microscopic approaches for studying kidney functions. Nephron Physiology. 2006; 103:p63–70. [PubMed: 16543769]
85. Yu W, Sandoval RM, Molitoris BA. Quantitative intravital microscopy using a Generalized Polarity concept for kidney studies. American journal of physiology Cell physiology. 2005; 289:C1197–208. [PubMed: 16033906]
86. Poreddy AR, Neumann WL, Freskos JN, et al. Exogenous fluorescent tracer agents based on pegylated pyrazine dyes for real-time point-of-care measurement of glomerular filtration rate. Bioorganic & medicinal chemistry. 2012; 20:2490–7. [PubMed: 22459210]
87. Rabito C, Halpern EF, Scott J, Tolkoff-Rubin N. Accurate, fast, and convenient measurement of glomerular filtration rate in potential renal transplant donors. Transplantation. 2010; 90:510–7. [PubMed: 20595931]
88. Rabito CA, Chen Y, Schomacker KT, Modell MD. Optical, real-time monitoring of the glomerular filtration rate. Applied optics. 2005; 44:5956–65. [PubMed: 16231803]
89. Rajagopalan R, Neumann WL, Poreddy AR, et al. Hydrophilic pyrazine dyes as exogenous fluorescent tracer agents for real-time point-of-care measurement of glomerular filtration rate. Journal of medicinal chemistry. 2011; 54:5048–58. [PubMed: 21667980]
90. Schock-Kusch D, Sadick M, Henninger N, et al. Transcutaneous measurement of glomerular filtration rate using FITC-sinistrin in rats. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2009; 24:2997–3001.
91. Schock-Kusch D, Xie Q, Shulhevich Y, et al. Transcutaneous assessment of renal function in conscious rats with a device for measuring FITC-sinistrin disappearance curves. Kidney international. 2011; 79:1254–8. [PubMed: 21368744]
92. Wang E, Meier DJ, Sandoval RM, et al. A portable fiberoptic ratiometric fluorescence analyzer provides rapid point-of-care determination of glomerular filtration rate in large animals. Kidney international. 2012; 81:112–7. [PubMed: 21881552]
93. Yu W, Sandoval RM, Molitoris BA. Rapid determination of renal filtration function using an optical ratiometric imaging approach. American journal of physiology Renal physiology. 2007; 292:F1873–80. [PubMed: 17311910]
94. Bivona BJ, Park S, Harrison-Bernard LM. Glomerular filtration rate determinations in conscious type II diabetic mice. American journal of physiology Renal physiology. 2011; 300:F618–25. [PubMed: 21147841]
95. Schock-Kusch D, Shulhevich Y, Xie Q, et al. Online feedback-controlled renal constant infusion clearances in rats. Kidney international. 2012; 82:314–20. [PubMed: 22513822]
96. Udy AA, Jarrett P, Stuart J, et al. Determining the mechanisms underlying augmented renal drug clearance in the critically ill: use of exogenous marker compounds. Critical care. 2014; 18:657. [PubMed: 25432141]
97. Zitta S, Schrabmair W, Reibnegger G, et al. Glomerular filtration rate (GFR) determination via individual kinetics of the inulin-like polyfructosan sinistrin versus creatinine-based population-derived regression formulae. BMC Nephrol. 2013; 14:159. [PubMed: 23876053]
98. Shmarlouski, A.; Schock-Kusch, D.; Shulhevich, Y., et al. A novel analysis technique for transcutaneous measurement of glomerular filtration rate with ultra-low dose marker concentrations. IEEE transactions on bio-medical engineering; 2015;
99. Vogt, R., Jr; Marti, GE.; Zenger, V. Standardization and Quality Assurance in Fluorescence Measurements I. Vol. 5. Springer; Berlin Heidelberg: 2008. p. 3-31.

100. Sambataro M, Thomaseth K, Pacini G, et al. Plasma clearance rate of 51Cr-EDTA provides a precise and convenient technique for measurement of glomerular filtration rate in diabetic humans. Journal of the American Society of Nephrology : JASN. 1996; 7:118–27. [PubMed: 8808118]

101. Sapirstein LA, Vidt DG, Mandel MJ, Hanusek G. Volumes of distribution and clearances of intravenously injected creatinine in the dog. The American journal of physiology. 1955; 181:330–6. [PubMed: 14376619]

102. Sheppard CWHA. The mathematical basis of the interpretation of tracer experiments in closed steady-state systems. J of Applied Physics. 1951; 22:510–20.

103. Schwartz GJ, Furth S, Cole SR, Warady B, Munoz A. Glomerular filtration rate via plasma iohexol disappearance: pilot study for chronic kidney disease in children. Kidney international. 2006; 69:2070–7. [PubMed: 16612328]

104. Work DF, Schwartz GJ. Estimating and measuring glomerular filtration rate in children. Current opinion in nephrology and hypertension. 2008; 17:320–5. [PubMed: 18408486]

105. Thomaseth K, Amici G. Optimal design of a two-sample test for assessing [125I]iothalamate plasma clearance in peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1998; 13:2265–70.

106. Earle KA, Mehrotra S, Dalton RN, Denver E, Swaminathan R. Defective nitric oxide production and functional renal reserve in patients with type 2 diabetes who have microalbuminuria of African and Asian compared with white origin. Journal of the American Society of Nephrology : JASN. 2001; 12:2125–30. [PubMed: 11562411]

107. Livi R, Teghini L, Pignone A, Generini S, Matucci-Cerinic M, Cagnoni M. Renal functional reserve is impaired in patients with systemic sclerosis without clinical signs of kidney involvement. Ann Rheum Dis. 2002; 61:682–6. [PubMed: 12117672]

Figure 1A

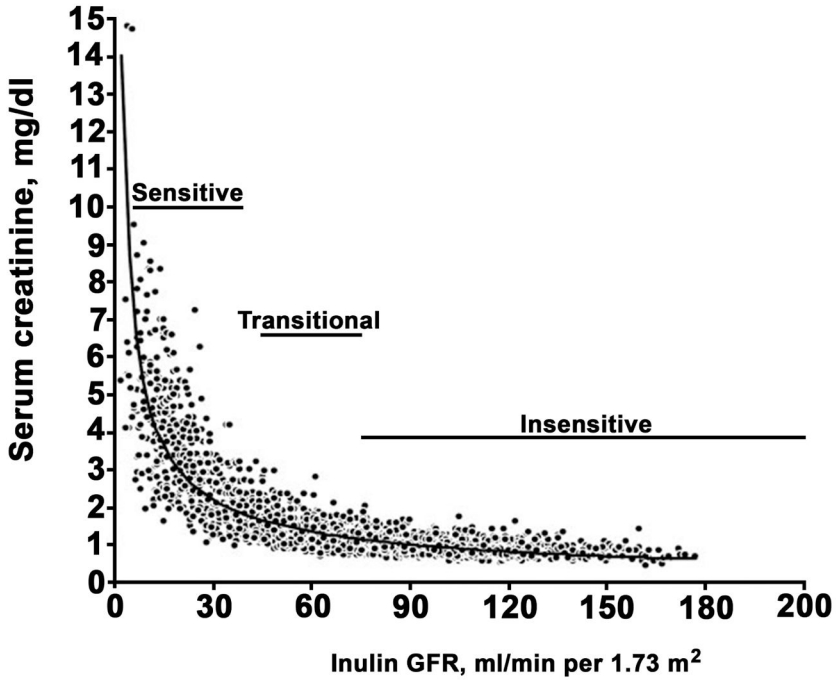

Figure 1B

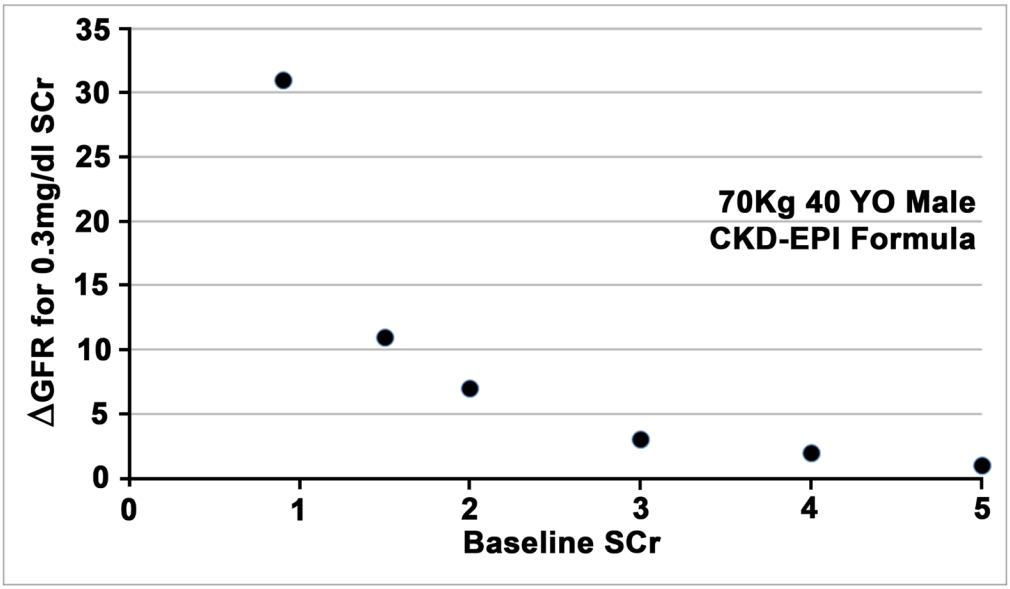

Figure 1.

Figure 1A. Serum creatinine vs inulin measured GFR adopted from Botev R et al.[24]

Figure 1B. Baseline serum creatinine of an idealized 70kg, 40 year old male vs the change in GFR that it would take to result in a 0.3mg/dl change in serum creatinine (the definition of AKI), using the CKD-EPI formula.

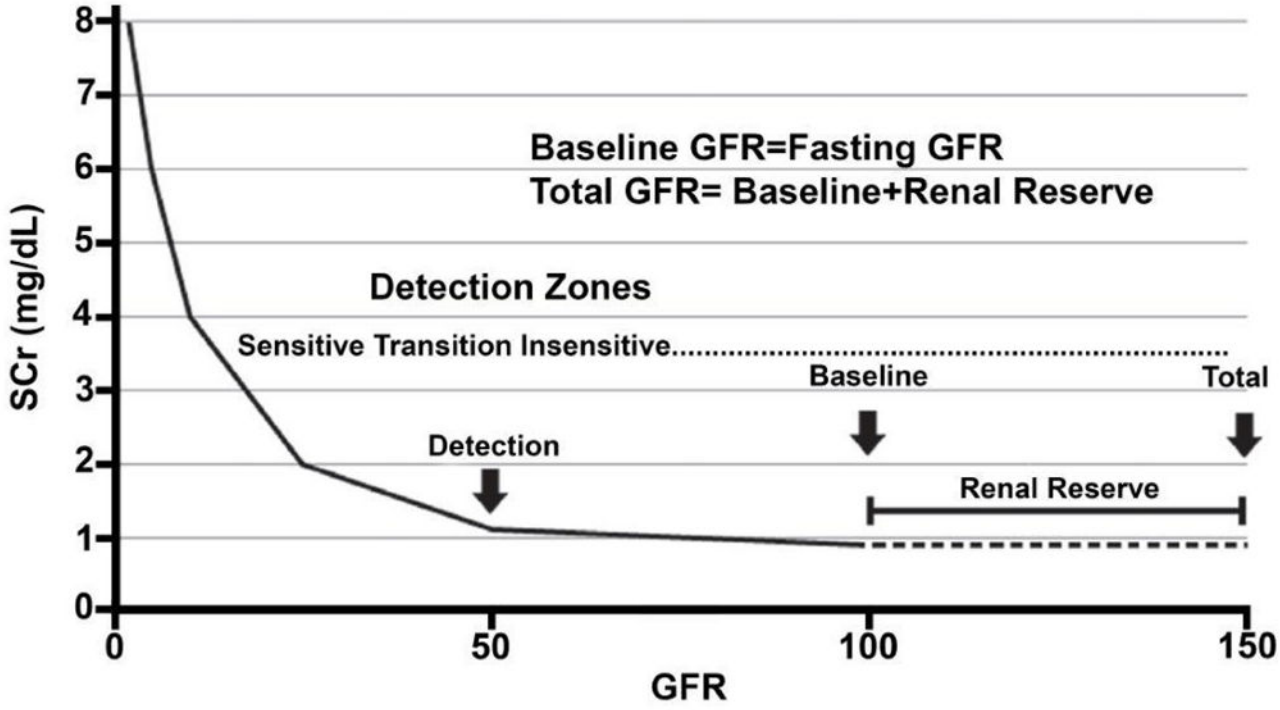

Figure 2.
Idealized GFR vs serum creatinine showing renal reserve in a patient with normal kidney function.

Table 1

Clinical Applications for Real-Time GFR

- Screen test for hospital-acquired kidney injury in patients with or without preexisting kidney damage
  - Diagnosis
  - Quantifying severity of injury
  - Asses therapeutic response
- Drug-dosage adjustments in patients with acute or chronic kidney dysfunction
- Risk assessment of AKI
- Rate of progression of kidney disease, quantify baseline, and renal reserve
- Monitor patients requiring repeated administration of nephrotoxic drugs
- Indirect assessment of visceral organ perfusion, such as during prolonged anesthesia
- Clinical trials for determining actual loss of total kidney function over a period of time
- Screening for hyperfiltration in diabetics to identify an early signal of pending CKD development

Table 2

Fluorescent Probe Determination of GFR

Molecular Marker	Sample Space	Detector Site	Readout	Species	Reference
Dextrans 150kDa-Rh 5kDa-FITC	Vasculature 2-Compartment Model	Venous	PV, GFR	Dogs	Wang, E et al AJP: 2010; Wang et al Kidney Inter: 2011
Sinistrin-FITC	ECF Single Compartment Model	Skin	Decay Rate	Rats, Rats	Schock-Kusch, D et al, Kidney Inter: 2011; Schock-Kusch, D et al NDT:2009
99mTc-DTPA	ECF Single Compartment Model	Arm	Decay Rate		Rabito, CA et al Clin and Trans Res: 2010
Carbustyril 124-DTPA-Eu	ECF Single Compartment Model	Skin	Decay Rate	Rats	Rabito, CA et al Applied Optics: 2005
Pyrazine – derivatives	ECF Single Compartment Model	Skin	Decay Rate	Rats, Rats	Poreddy, AR et al Bioorganic & Medicinal Chem: 2012; Rajagopalan, R et al Medicinal Chemistry; 2011
Inulin-FITC	Vasculature 2-Compartment Model	Venous	GFR		Qi, Z et al AJP-Renal: 2004
Inulin-FITC	Vasculature And Urine	Venous	PV, GFR	Mice	Bivona, B et al AJP-Renal: 2011
Sinistrin-FITC	ECF Continuous Infusion	Skin	Decay Rate	Rats	Schok-Kusch, D et al Kidney Inter: 2012
Sinistrin-FITC	ECF Single Compartment Model	Skin	Decay Rate	Rats	Shmarlouski, A et al TBME: 2015